

An antioxidant function for DMSP and DMS in marine algae

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The algal osmolyte dimethylsulphoniopropionate (DMSP) and its enzymatic cleavage product dimethylsulphide (DMS) contribute significantly to the global sulphur cycle^{1–3}, yet their physiological functions are uncertain⁴. Here we report results that, together with those in the literature^{5,6}, show that DMSP and its breakdown products (DMS, acrylate, dimethylsulphoxide, and methane sulphinic acid) readily scavenge hydroxyl radicals and other reactive oxygen species, and thus may serve as an antioxidant system, regulated in part by enzymatic cleavage of DMSP. In support of this hypothesis, we found that oxidative stressors, solar ultraviolet radiation⁷, CO₂ limitation⁸, Fe limitation, high Cu²⁺ (ref. 9) and H₂O₂ substantially increased cellular DMSP and/or its lysis to DMS in marine algal cultures. Our results indicate direct links between such stressors and the dynamics of DMSP and DMS in marine phytoplankton, which probably influence the production of DMS and its release to the atmosphere. As oxidation of DMS to sulphuric acid in the atmosphere provides a major source of sulphate aerosols and cloud condensation nuclei³, oxidative stressors—including solar radiation and Fe limitation—may be involved in complex ocean–atmosphere feedback loops that influence global climate and hydrological cycles^{1,2}.

DMSP occurs at high cellular concentrations (100–400 mmol l^{−1}) in many marine algae, including prymnesiophytes and dinoflagellates, and thus functions as an osmolyte in these algae. DMSP has also been proposed to serve as a cryoprotectant in polar algae and as a grazing deterrent via its cleavage to acrylate, although its overall physiological functions remain unclear⁴. Its cellular concentration increases with light in many algal species^{10,11}, an effect not readily explained by existing proposed functions. In laboratory experiments, we found that DMSP reacts rapidly with the hydroxyl radical (·OH), and thus could serve as an effective cellular scavenger of this harmful radical (Table 1). DMSP's enzymatic cleavage products, DMS and acrylate, are even more effective at scavenging ·OH, as are the DMS oxidation products dimethylsulphoxide (DMSO) and methane sulphinic acid (MSNA) (Table 1). Calculations suggest that, taken together, these molecules constitute an antioxidant system, which could be more effective at scavenging ·OH in high-DMSP algae than other well-recognized antioxidants, such as ascorbate and glutathione (Table 1). An antioxidant function would explain the observed increase in algal DMSP concentrations at high light^{10,11}, as ·OH is produced as a by-product of photosynthesis.

All five proposed antioxidant compounds—DMSP, acrylate, DMS, DMSO and MSNA (Table 1)—have unique chemical and physical properties that the cell can exploit. The metabolically compatible zwitterion DMSP is lysed to DMS and acrylate via the enzyme DMSP lyase, whose cellular function and regulation are not well understood¹². Acrylate and DMS are ~20 and ~60 times more reactive towards ·OH than is DMSP (Table 1), and DMS is highly reactive toward singlet oxygen¹³. Acrylate is charged at physiological pHs, and like DMSP, cannot penetrate into lipid membranes; but uncharged DMS molecules can readily diffuse into photosynthetic

membranes, an important site of harmful lipid peroxidation reactions. Thus, DMSP lysis should substantially increase the antioxidant protection in both aqueous and lipid membrane phases within the cell. This increased protection could be modulated by DMSP lyase, providing an important metabolic function for this enzyme.

Much of the released DMS should oxidize to DMSO or other oxidized sulphur species, while the remainder would diffuse from the cell. The DMSO produced from ·OH oxidation of DMS or DMSP (see Methods) is much more hydrophilic than DMS, retarding its loss across cell membranes and allowing it to accumulate at high cellular concentrations^{14–16}. These high concentrations, combined with a high reactivity toward ·OH, makes DMSO an effective antioxidant (Table 1), as has been proposed previously¹⁵ and has been directly observed in isolated chloroplasts⁶. DMSO oxidation by ·OH produces the water-soluble antioxidant MSNA, which can further react with and scavenge ·OH¹⁷ (Table 1). Thus, enzymatic lysis of DMSP gives rise to four water- or lipid-soluble antioxidant scavengers of ·OH radicals and at least one scavenger (DMS) of singlet oxygen. We suggest that this multiplicative effect combined with high cellular DMSP concentrations make DMSP and its lysis and oxidation products a highly effective antioxidant system.

Organisms generally acclimate to oxidative stress by up-regulation of antioxidant systems^{7–9}. Thus, under our hypothesis, phytoplankton should respond to higher levels of oxidative stress by increased DMSP synthesis and lysis to DMS. This indeed is what we observed for the diatom *Thalassiosira pseudonana* under CO₂ limitation, a known oxidative stressor⁸, and Fe limitation, shown here to also oxidatively stress cells. A nutrient-sufficient control culture had a high specific growth rate (1.45 d^{−1}), high cellular chlorophyll *a*, low cellular DMSP (~1 mmol l^{−1}), low DMS to cell-volume ratios, and low cellular activity of the antioxidant enzyme ascorbate peroxidase (APX; Table 2). But under CO₂ limitation and Fe limitation, we observed 20- to 60-fold increases in cellular DMSP, increased DMS/cell-volume ratios, and increased APX activities, in conjunction with large decreases in growth rate and chlorophyll *a*. APX is the main chloroplast enzyme for removal of H₂O₂, a reactive by-product of photosynthesis and a major precursor for ·OH radical formation^{18,19}. As antioxidant systems are up-regulated under increased oxidative stress, the significant increase in APX activity/chlorophyll *a* ratios under CO₂ limitation ($P < 0.001$, *t*-test) and Fe limitation ($P < 0.01$) indicate that both increased oxidative stress within the chloroplast. The increase in APX activity is noteworthy as APX contains Fe, and would be expected to decrease under Fe limitation. But under Fe limitation, there is a decrease in Fe-containing components of the photosynthetic electron transport chain (for example, the cytochrome *b₆/f* complex) relative to

Table 1 Computed *in vitro* half-lives for ·OH radical

Compound	Concentration (mmol l ^{−1})	Rate constant* (M ^{−1} s ^{−1})	Ref.	·OH half-life (ns)
DMSP	150–450†	3 × 10 ⁸	This work	5–15
Acrylate	~1–40?	5.6 × 10 ⁹	Ref. 5	3–124
DMS	~1–40?	1.9 × 10 ¹⁰	Ref. 5	0.9–36
DMSO	30–90†	6.6 × 10 ⁹	Ref. 5	1.2–3.5
MSNA	?	9 × 10 ⁹	This work	?
Glutathione	2.4‡	1.4 × 10 ¹⁰	Ref. 5	21
Ascorbate	1–6§	1.1 × 10 ¹⁰	Ref. 5	11–63

Data are shown for a range of concentrations of DMSP and its breakdown products, and of antioxidants (glutathione and ascorbate) in high-DMSP algae (for example, *E. huxleyi*).

*Rate constant for reaction of each compound with ·OH at 25 °C; pH conditions are 7.5 for this work, and 7.0 for ref. 5.

†Approximate DMSP range that we observed in *E. huxleyi*. The cellular DMSO concentration was computed by dividing the cell DMSP concentration by the DMSP/DMSO ratio (~5) found in near-surface oceanic plankton¹⁶.

‡Measured value in *E. huxleyi* (CCMP373) cultures²⁹.

§Estimated average range for phytoplankton based on the reported range per dry weight of algal cells (5–28 mmol g^{−1}; ref. 30), an assumed algal dry to wet weight ratio of 0.8, and a cell density of 1.03 g cm^{−3} (the value for sea water).

Table 2 Effect of CO₂ limitation and Fe limitation in *T. pseudonana* cultures

Treatment	Day	Growth rate (d ⁻¹)	Cell vol. (μl l ⁻¹)*	pH	[CO ₂] (μmol l ⁻¹)	Cell chl <i>a</i> (mmol l ⁻¹)†	Cell DMSP (mmol l ⁻¹)‡	DMS (mmol l ⁻¹)‡	APX act. (per l CV)‡	APX act. (per mol chl)‡
Control	9	1.45	6.6	ND§	ND	4.0	0.88 ± 0.02	0.08 ± 0.1	118 ± 37	29 ± 9
	12	1.45	7.8	8.42	5.1	4.4 ± 0.2	1.46 ± 0.01	0.11 ± 0.0	99 ± 24	23 ± 6
	13	1.45	4.9	8.32	7.0	3.9 ± 0.0	0.85 ± 0.01	0.0	93 ± 18	24 ± 5
CO ₂ -limited	6	0.31	35.6	9.00	0.69	3.0 ± 0.1	18.9 ± 0.2	0.42 ± 0.01	255 ± 13	84 ± 4
	7	0.31	37.8	9.10	0.47	2.9 ± 0.0	13.5 ± 0.2	0.32	209 ± 20	72 ± 7
	10	0.15	39.8	9.26	0.25	1.9 ± 0.0	59.6 ± 1.7	0.59 ± 0.01	305 ± 23	160 ± 12
Fe-limited	20	0.18	46.5	9.40	0.14	1.3 ± 0.0	64.2 ± 0.6	0.42 ± 0.01	226 ± 4	173 ± 3
	13	0.04	2.6	8.30	7.4	2.1 ± 0.2	6.5 ± 0.7	0.0	134 ± 14	64 ± 7
	14	0.04	1.8	8.27	8.1	1.7 ± 0.0	7.6 ± 0.0	0.35	458 ± 9	269 ± 5
	25	0.04	1.5	8.28	7.9	1.3	20.5 ± 5	0.83 ± 0.07	ND	ND

*μl of cell volume per litre of culture.

†mmol of cellular DMSP, chlorophyll *a* (chl *a*), or total DMS per litre of cell volume. Total DMS was measured in unfiltered culture samples.

‡mmol ascorbate consumed per minute per litre cell volume (CV) or mol ascorbate per mol chl *a* per minute, respectively.

§ND: not determined.

||Mean ± range of two replicates for chl *a*, DMSP and DMS. APX activities are given as the mean ± s.d. for 2–4 replicates (mean 3.2).

chlorophyll *a*²⁰, which leads to inefficient photosynthetic electron transport²⁰. This, in turn, should increase oxidative stress¹⁹, which if not alleviated, could lead to severe cell damage or death.

Similar effects of CO₂ and Fe limitation were observed with other species. In the coccolithophore *Emiliania huxleyi*, which has constitutively high DMSP, CO₂ limitation caused up to a 150% increase in cellular DMSP and large relative increases in the DMS to cell-volume ratio (Table 3). For the coastal diatom *Skeletonema costatum*, Fe limitation caused a 67% decrease in growth rate and a three-fold increase in the cell DMSP/C ratio (from 1.6 to 4.7 mmol mol⁻¹) (W.S., D.J.K., R.P.K. and S.H., unpublished data). Fe limitation also has been found to increase cellular DMSP concentrations in *Phaeocystis* sp.¹¹.

The response of *E. huxleyi* to other oxidative stressors was also determined. Initial experiments examined the effect of solar UV radiation, an important environmental stress⁷. Growth of *E. huxleyi* cultures under 30% solar radiation, with different portions of the UV spectrum removed with cut-off filters, increased DMSP/cell-volume ratios by 5–100% and DMS/cell volume ratios by 2.4- to 36-fold relative to values in a culture grown under a similar intensity of fluorescent light with essentially no UV radiation (Table 4). The largest effect occurred in sunlight attenuated with Mylar or polycarbonate, which remove UV-B (290–320 nm) radiation but allow most UV-A (320–400 nm) to pass. Smaller increases occurred under full spectral sunlight or sunlight attenuated with UV-adsorbing glass, which removes >90% of the UV at wavelengths <383 nm. Exposure to UV oxidative stress should both promote metabolic induction of antioxidant systems⁷ and consume cellular antioxidants (DMSP and DMS). In addition, photolysis by UV in sea water can degrade DMS released from cells²¹. Consequently, the DMS measured under high levels of UV exposure may substantially underestimate overall DMS production. Because of these effects, the highest DMS accumulation should occur at intermediate levels of UV stress, as observed here in the Mylar and polycarbonate treatments.

In further support of our antioxidant hypothesis, the exposure of *E. huxleyi* to two additional oxidative stressors (high Cu²⁺ and H₂O₂) increased DMS/cell-volume ratios 9 to 15-fold (see Supplementary Information). Copper catalyses the reduction of H₂O₂ to ·OH radicals, and is a known oxidative stressor in phytoplankton⁹.

Our results showing increased ratios of DMS and DMSP to chlorophyll *a* with exposure to solar UV radiation (Table 4) are consistent with field data. Seasonally, maximum DMS concentrations in oceanic surface waters occur in the summer when solar UV exposure is highest owing to higher UV intensity, longer photoperiods, and shallower mixed layer depths^{22,23}. Surface DMS/chlorophyll *a* ratios increase by 10–50-fold from winter to summer in a broad range of subtropical, temperate and sub-arctic waters. This seasonal pattern has been suggested to be related to UV

photo-inhibition of bacteria which use DMS as a growth substrate²² and fluctuations in DMS photolysis rates²³; however, our results indicate that this pattern may also be due to higher algal DMSP production and increased lysis to DMS with higher solar UV stress. Much of the seasonal pattern may be related to the selection of high-DMSP species during the summer, capable of growth under high exposure to solar irradiance, such as *E. huxleyi*²⁴. But we argue that the high DMSP levels may help these species to adapt to the high summertime oxidative stress. Ratios of DMS, DMSP and DMSO to chlorophyll *a* are usually highest near the sea surface and decrease substantially with depth in conjunction with solar attenuation^{25,26}. Furthermore, in the euphotic zone of the tropical Atlantic, particulate DMSP (size range 0.7–10 μm) was closely correlated with levels of antioxidant carotenoids²⁶, again suggesting a direct linkage between oxidative stress and cellular levels of DMSP.

Linkages between solar UV radiation and algal DMSP and DMS production have important implications for large-scale feedback interactions between marine phytoplankton and atmospheric chemistry and physics^{1–3}. In these interactions, DMS diffuses into the atmosphere where it is oxidized to acidic species (for example, H₂SO₄) that form aerosols which serve as cloud condensation nuclei. The resultant cloud droplets increase solar reflectance, which has a cooling effect on the planet^{1,2}. There is an inherent negative feedback at work, because increased solar irradiance is associated with higher DMS concentrations at the ocean's surface^{1,22}, which our experiments suggest is at least partly linked to increased photo-oxidative stress. The resultant higher DMS flux to the atmosphere should increase the formation of acidic sulphur aerosols and clouds, thereby reducing solar inputs to the ocean's surface, which should have a cooling effect^{1,2} and reduce biological solar stress. Our results suggest that solar irradiance and other oxidative stressors, such as Fe limitation or CO₂ limitation, may act

Table 3 Effect of CO₂ limitation in cultures of *E. huxleyi*

Treatment	Day*	Growth rate (d ⁻¹)	Cell vol. (μl l ⁻¹)	pH	[CO ₂] (μmol l ⁻¹)	DMS/CV (mmol l ⁻¹)†	Cell DMSP (mmol l ⁻¹)‡
Control	10	0.73	8.1	8.46	4.6	0.8 ± 0.0	185 ± 0
	11	0.73	4.6	8.40	5.5	0.3 ± 0.0	167 ± 3
	12	0.73	4.9	8.41	5.3	0.1 ± 0.0	200 ± 10
	13	0.73	5.9	8.46	4.6	0.0 ± 0.0	193 ± 6
CO ₂ -limited	11	0.23‡	21.9	8.97	0.77	0.8 ± 0.0	232 ± 8
	12	0.23	21.5	8.92	0.93	1.1 ± 0.1	267 ± 6
	14	0.23	26.0	9.07	0.52	2.0 ± 0.2	302 ± 21
	17	0.09‡	29.6	9.26	0.25	4.7 ± 0.2	365 ± 10
	18	0.09	29.9	9.20	0.31	6.3 ± 0.1	396 ± 11
	19	0.09	29.4	9.18	0.34	5.5 ± 0.2	478
	20	0.09	30.3	9.14	0.40	5.3 ± 0.3	461 ± 17

*Days after culture inoculation.

†mmol total culture DMS or cellular DMSP per litre of cell volume. Data are given as the mean ± range for two replicates.

‡CO₂ limitation of growth rate was first evident on day 11. 0.23 d⁻¹ is the average specific growth rate for days 11–14 while 0.09 d⁻¹ is the average rate for days 14–20.

Table 4 Effect of solar radiation on DMSP and DMS in cultures of *E. huxleyi*

Treatment	Growth rate (d ⁻¹)	DMS/CV (mmol l ⁻¹) ^a	DMSP/CV (mmol l ⁻¹) ^a	chl a/CV (mmol l ⁻¹)	DMSP/chl a (mol mol ⁻¹)
Fluorescent light†	0.66	1.0 ± 0.0 (2)‡	198 ± 7 (2)	2.8	70
Exp. 1; 30% sun, UV A + B filter	0.64	4.7 ± 0.1 (2)	208 ± 5 (2)	3.2	65
Exp. 1; 30% sun, Mylar (UV-B filter)	0.57	36.2 ± 1.3 (2)	393 ± 6 (2)	3.9	101
Exp. 1; 30% sun, Full UV	0.65	2.4 ± 0.1 (2)	315 ± 1 (2)	3.6	86
Exp. 2; 30% sun, polycarbonate§	0.68	16.3 ± 0.9 (3)	274 ± 17 (4)	2.75 ± 0.02 (2)	96
Exp. 2; 10% sun, polycarbonate§	0.68	6.8 ± 0.6 (4)	256 ± 4 (4)	4.05 ± 0.08 (2)	63

^ammol of total culture DMS or DMSP normalized to cell volume (CV). All indoor and outdoor measurements were made 5–6 h into a 14-h light period.

†14 h d⁻¹ of fluorescent lighting at a PAR intensity of 800 μmol m⁻² s⁻¹.

‡The number of replicate measurements is given in parenthesis. The ± value denotes the range for *n* = 2 and s.d. for *n* > 2.

§Absorbs UV-B and >50% of UV-A at wavelengths <350 nm.

in concert as controls on DMS release from the ocean's surface, with important feedbacks on both climate and the productivity and composition of phytoplankton communities. □

Methods

Culture experiments

The CO₂ and Fe limitation experiments with *T. pseudonana* (clone CCMP1335) and *E. huxleyi* (CCMP374) were conducted at 20 °C under fluorescent light (Vita-Lite Plus, Durotest; intensity of 500 μE m⁻² s⁻¹). *T. pseudonana* and *E. huxleyi* were grown under light:dark cycles of 12 h:12 h and 14 h:10 h, respectively. Axenic cultures were grown in tightly sealed polycarbonate bottles with <5% headspace to minimize CO₂ and DMS exchange with the atmosphere. The culture media consisted of filtered sea water²⁷ enriched with 160 μM NaNO₃, 10 μM Na₂HPO₄, 40 μM Na₂SiO₃, 10 nM Na₂SeO₃, and vitamins (0.37 nM B₁₂, 2 nM biotin and 300 nM thiamin) and a 0.1 mM EDTA-trace-metal ion buffer system²⁷. The nitrate, phosphate and vitamins were five times normal levels²⁷ to ensure growth limitation by CO₂ at high biomass rather than by N and P. For the control and CO₂ limitation treatments, a non-limiting Fe level (1 μM)²⁷ was added.

The cells were grown semi-continuously by harvesting a portion of the culture periodically (usually every 1–3 d) and replacing it with fresh medium. For the control, cell volumes were maintained at ≤8 μl per litre of culture to avoid nutrient limitation. To achieve CO₂ limitation, cultures were grown to high cell volumes, which resulted in up to a 40-fold decrease in CO₂ concentrations (computed from the CO₂ alkalinity, pH and conditional constants for CO₂ and bicarbonate dissociation). In the Fe-limited *T. pseudonana* treatment, cells were transferred from an Fe-sufficient medium (1 μM Fe) to a medium with no added Fe and grown into Fe limitation. The harvested portions of all cultures were analysed for total cell volume²⁷, pH, cellular chlorophyll *a*²⁷, total DMS²⁸ and particulate (that is, cellular) DMSP²⁸. In addition, APX activity was measured¹⁸ in *T. pseudonana*. For control and Fe-limited cultures, the specific growth rate was computed by linear regression of ln cell volume versus time²⁷ after accounting for culture dilution effects.

The two solar exposure experiments (Table 4) were conducted with axenic cultures of *E. huxleyi* (CCMP374) grown in seawater media similar to the above, but with five-fold lower NaNO₃ (32 μM), Na₂HPO₄ (2 μM) and vitamins. In addition, the metal ion buffering agent was switched from 0.1 mM EDTA to 0.1 mM NTA (see Supplementary Information) to avoid photolysis of FeEDTA²⁷. The experiments were initiated by growing a non-limited control culture and two experimental cultures under 14 h d⁻¹ of fluorescent light (Cool White, Philips). The light intensity was 800 μE m⁻² s⁻¹, approximating that of 30% solar radiation (see below). The experimental cultures were transferred to media held in 80-ml sealed quartz tubes (exp. 1) or in 150-ml polycarbonate plastic bottles (exp. 2) all with minimal headspace. The cultures were exposed to sunlight attenuated to 30% (exp. 1) and to 30% and 10% (exp. 2) using neutral density screens. In addition, two of the three quartz tubes in exp. 1 were covered with a UV cut-off filter: Mylar, which absorbed >90% of solar UV radiation at wavelengths <317 nm, or UV-absorbing glass, which absorbed >90% of radiation at wavelengths <383 nm. The polycarbonate bottles constitute a short-wavelength UV filter as they absorb >50% of UV radiation at wavelengths <350 nm, and >90% at wavelengths <330 nm. In exps 1 and 2 the cultures experienced three- and five-day growth lags, respectively, while acclimating to the new light conditions, and then grew exponentially. Total DMS²⁸, total DMSP²⁸, cell volume²⁷ and cell chlorophyll *a*²⁷ were measured in the cultures after three days of exponential growth in exp. 1 and five days in exp. 2. The same measurements were made after 10 d of exponential growth in the control. Specific growth rates in these cultures were measured²⁷ during the exponential growth period. The two solar experiments were conducted at ~23 °C in a flowing water bath under mostly sunny skies during May 2001 (latitude 35° N). Sterile technique was used in all experiments (Tables 2–4) to avoid bacterial contamination (see Supplementary Information).

In early tests, the measured cell DMSP concentration decreased with increasing volume filtered (1–10 ml) in *E. huxleyi* under gentle vacuum (<5 mm Hg), indicating some loss of DMSP during filtration due to cell lysis. This problem did not occur with *T. pseudonana*, which has a robust silica frustule. Because of these problems with *E. huxleyi*, filtration was avoided and total culture DMSP was measured in early experiments with this species, including the solar exposure experiment (Table 4). Recently, we found that loss of cell DMSP can be largely eliminated by using gravity filtration and a small filtration volume (1–2 ml). This procedure was used to determine cellular DMSP in the CO₂-limitation experiment with *E. huxleyi* (Table 3). In this experiment, DMSP concentrations measured in the culture filtrates (data not shown) were 2.3 ± 0.7% (*n* = 8) and 2.6 ± 1.2%

(*n* = 12) of the total culture DMSP for the control and CO₂-limited treatments. Thus, virtually all of the DMSP is present in the cells. Furthermore, the large variability in DMSP in the filtrates (up to 2.8-fold between duplicate filtrates) may be due to a variable residual lysis of cells under even these gentlest of filtration conditions.

Hydroxyl radical reaction with DMSP, DMS, DMSO and MSNA

Laboratory experiments at 25 °C and pH 7.5 confirmed that •OH radicals oxidize DMS to DMSO, MSNA and methane sulphonate (MSA), and quantitatively oxidize DMSO stepwise to MSNA and MSA. DMSP was likewise oxidized quantitatively to MSNA and MSA, with DMSO as a likely reactive intermediate. In these experiments, rate constants for reactions of DMSP and MSNA with the •OH radical were also measured by comparison with the reaction kinetics of DMSO under identical conditions. The rate constant of DMSO with •OH under these conditions was 6.6 × 10⁹ M s⁻¹ (ref. 5). To generate the •OH radical, a 10 mM aqueous solution of H₂O₂ in 10 mM phosphate buffer (pH 7.5) was irradiated under UV lamps (UV-A, Q-Panel) with UV-A and UV-B irradiances of 3.0 and 0.28 μW cm⁻². Loss of DMSP and DMSO were measured over time with gas chromatography²⁸, and loss of MSNA and its associated conversion to MSA were measured by ion chromatography. Rate constant calculations were corrected for direct oxidation by H₂O₂ measured in parallel solutions in the dark. Nearly identical results were obtained when nitrate photolysis was used to generate •OH.

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Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum*

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Widespread use of antimalarial agents can profoundly influence the evolution of the human malaria parasite *Plasmodium falciparum*. Recent selective sweeps for drug-resistant genotypes may have restricted the genetic diversity of this parasite, resembling effects attributed in current debates^{1–4} to a historic population bottleneck. Chloroquine-resistant (CQR) parasites were initially reported about 45 years ago from two foci in southeast Asia and South America⁵, but the number of CQR founder mutations and the impact of chloroquine on parasite genomes worldwide have been difficult to evaluate. Using 342 highly polymorphic microsatellite markers from a genetic map⁶, here we show that the level

of genetic diversity varies substantially among different regions of the parasite genome, revealing extensive linkage disequilibrium surrounding the key CQR gene *pfcr7* and at least four CQR founder events. This disequilibrium and its decay rate in the *pfcr7*-flanking region are consistent with strong directional selective sweeps occurring over only ~20–80 sexual generations, especially a single resistant *pfcr7* haplotype spreading to very high frequencies throughout most of Asia and Africa. The presence of linkage disequilibrium provides a basis for mapping genes under drug selection in *P. falciparum*.

We examined microsatellite markers covering the 14 haploid chromosomes, at an average interval of ~75 kilobases (kb), from 87 *P. falciparum* worldwide isolates (Supplementary Information Fig. 1). Eighty-five per cent of the markers are highly polymorphic with 10 or more alleles. To evaluate whether genotypes reflect the geographical origins of the isolates, we tested the hypothesis of no allele sharing within a continental subset of isolates (Fig. 1a). The American isolates show very significant allele sharing for all chromosomes ($P = 1.5 \times 10^{-37}$ genome-wide), as do Asian isolates for most chromosomes except 1, 2, 8 and 14 ($P = 2.15 \times 10^{-7}$). In contrast, African isolates are very diverse and have a level of allele sharing similar to the bootstrap controls. This analysis adds genome-wide support to previous claims^{8–10} that distinct parasite population structures exist on different continents, and that African parasites could be the common ancestors of *P. falciparum* parasites worldwide because of their higher diversity.

However, substantial differences in allelic diversity exist among chromosomes (Fig. 1a), possibly reflecting recent directional selection superimposed on the patterns of geographical diversity. The CQR isolates from Africa and Asia shared many more alleles on chromosome 7 (where *pfcr7* is located) than the chloroquine sensitive (CQS) isolates (Fig. 1b), suggesting common origins for CQR parasites. To further explore the extent of allele sharing on chromosome 7, we analysed the *pfcr7* alleles and flanking

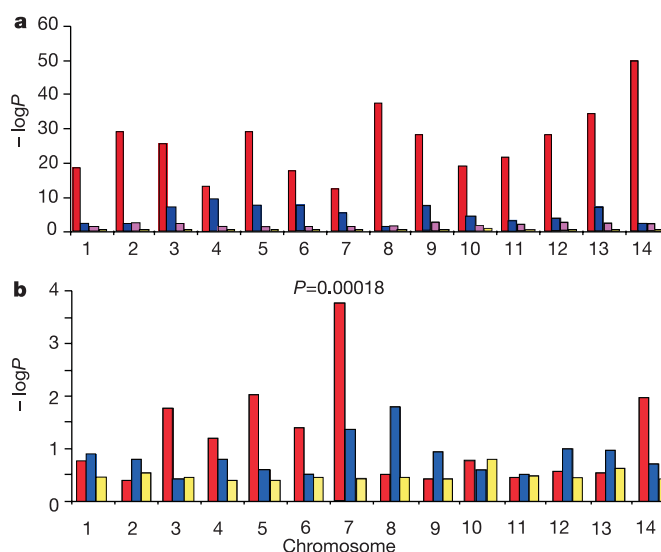


Figure 1 Genome-wide allele sharing analysis of *P. falciparum* isolates from various geographical regions or CQR/CQS (chloroquine-resistant/chloroquine sensitive) subsets. For the individual chromosomes, bars represent the negative $\log_{10}(P)$ values for obtaining by chance the amount of allele sharing observed, based on 20,000 bootstrap samples (see Methods; higher bars indicate more significant allele sharing). **a**, Allele sharing among parasites from Asia (blue), Africa (purple) and South America (red). Yellow, bootstrap controls. **b**, Higher allele sharing among CQR (red) than CQS (blue) parasites from Africa and Asia for chromosome 7 ($P = 1.76 \times 10^{-4}$) and to a lesser extent for chromosomes 3, 5 and 14. Yellow, bootstrap controls.